Epithelial Sensing of Fungal Invasion

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The mechanisms discriminating mucosal colonization from invasion by *Candida albicans* are unclear. In this issue, Moyes et al. show that epithelial cells discriminate between colonizing yeasts and invading hyphae by mechanisms involving c-Fos and MKP1 phosphatase, opening up new avenues for understanding the pathophysiology of mucosal fungal infections.

Candida albicans is a common opportunistic fungal pathogen that colonizes healthy human mucosa and skin, with approximately one-third of individuals being colonized at any given time (Perlroth et al., 2007). At the intestinal or vaginal mucosa, a complex system of recognition and host defense ensures efficient elimination of the microorganisms if they attempt to invade the host tissue (Romani, 2004). As a result of these defensive mechanisms, *Candida* colonizes the host but is prevented from invading the tissue unless the mucosal or skin barriers are breached or the immune system is compromised, which then leads to invasive or even disseminated *Candida* infection. In a healthy individual, *Candida* colonizing the mucosa does not induce an inflammatory reaction, whereas a protective inflammatory response is triggered upon tissue invasion by *C. albicans* (Netea et al., 2008). The mechanisms that allow the tolerance of colonizing yeasts but activate a defense response once the mechanical barriers are breached and *Candida* attempts to invade the tissue have remained obscure.

The study reported in this issue of *Cell Host and Microbe* by Moyes and colleagues provides an important first step in deciphering these mechanisms (Moyes et al., 2010). In their study, the authors attempt to understand how epithelial cells, the cell type in closest contact with mucosal or skin flora, are able to distinguish between colonizing *C. albicans* that poses no apparent threat and the invading *Candida* that can result in mucosal or systemic infection. The importance of epithelial cells in antifungal host defense is supported by clinical arguments (Perlroth et al., 2007), as well as by experimental studies that have described the mechanisms through which epithelial cells can activate immune cells such as neutrophils (Weindl et al., 2007). In the present study, Moyes at al. go a step further and identify fungal germination and the recognition of hyphae by the epithelial cell as a major danger signal indicating tissue invasion. This may seem hardly surprising, considering the solid literature implicating morphologic switch as a major virulence factor of *C. albicans* (Lo et al., 1997), but it is for the first time that the differential recognition of yeasts and hyphae is documented as a major discriminatory mechanism in sensing tissue invasion.

Pursuing the molecular mechanisms that allow the discrimination between yeasts and hyphae, the authors identify several crucial signaling pathways responsible for stimulation of cytokine production in epithelial cells by *C. albicans*. Both yeasts and hyphae induce the activation of the NF-kB pathway that is indispensable, but not sufficient, for the stimulation of cytokine production. In addition, both morphological forms induce a first phase activation of the transcription factor c-Jun through the MAPK p38, which is likely dependent on fungal cell wall recognition. However, a second phase of the transcription factor c-Fos and MAPK phosphatase MKP1 activation through p38 and ERK1/ERK2 kinase pathway is necessary for the induction of a cytokine response by epithelial cells, and this second phase can be induced only by *C. albicans* hyphae (Figure 1). Of interest, whereas c-Fos stimulates cytokine production, the activation of MKP1 seems to exert mainly regulatory functions on the p38 (and JNK) pathway. A secondary signal inducing cytokine production is represented by the fungal load, an increase of which can further stimulate induction of cytokines by the epithelial cells.

These findings have important conceptual and practical consequences. First, they describe a molecular mechanism that allows the epithelial cells to discriminate between invasive potentially pathogenic hyphae and harmless colonizing *C. albicans* yeasts. The proper discrimination between these two states is crucial for avoiding inappropriate inflammation yet responding promptly in case of fungal invasion. The in vivo relevance of the Moyes et al. finding is strengthened by their data showing increased expression of c-Fos and MKP1 in the mucosa of two patients with mucosal *C. albicans* infection. In addition, this model may be important for the recognition of other colonizing/invading microorganisms such as gut bacteria, although this remains to be demonstrated. Finally, these findings may provide clues about the mechanisms by which tolerance for colonizing *Candida* may be lost during inflammatory bowel disease. Specific anti-*Candida* antibodies (ASCA) have been described in patients with Crohn's disease (Standaert-Vitse et al., 2009), and *C. albicans* exacerbates colitis in mice (Jawhara et al., 2008). Disturbances in the mechanisms that normally function to tolerate colonizing yeasts may underlie intestinal autoinflammatory diseases.

This study is an important step in understanding the discrimination between colonization with yeasts and invasion by hyphae, yet important questions remain. First, what are the receptors of the endothelial cell responsible for this discriminatory recognition of yeasts and hypahe? The authors propose that a yet to be described receptor may be involved,

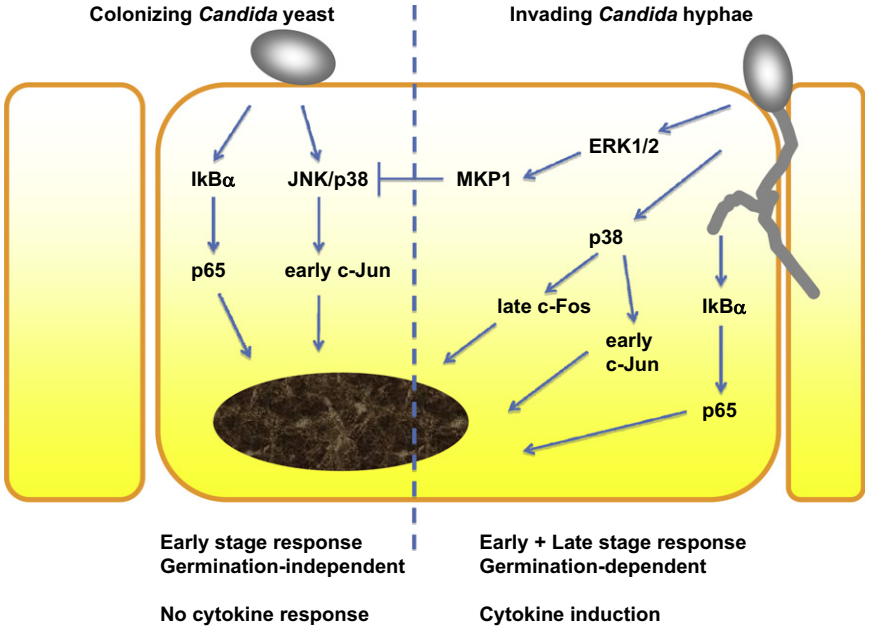

Figure 1. Differential Pathways of Epithelial Cell Activation by Colonizing Yeasts and Invading Hyphae

Yeasts induce the NF-κB subunit p65 and early c-Jun activation in response to the yeast form of *Candida albicans*, which does not induce a cytokine response. In contrast, hyphae stimulate p65 and the early c-Jun activation, as well as the MAPK phosphatase MKP-1 and the late c-Fos response, resulting in a potent cytokine release.

as no role for the known fungal pattern recognition receptors, including dectin-1, MR, TLR2, and TLR4, could be demonstrated. Second, which are the hyphal components that are differentially recognized? The argument that the polysaccharides of the fungal cell wall are not involved may not be definitive, as stimulation of cells with purified components in solution differ greatly from their steric presentation in the complex structure of the particulate cell wall. Finally, a still unanswered question of crucial importance relates to the identity of the mechanisms allowing the immune cells such as tissue macrophages and DCs that also sample the content of the colonizing microorganisms of the mucosa to discriminate between yeasts and hyphae. Immune cells recognize fungi mainly through pathogen recognition receptors such as Toll-like receptors and C-type lectin receptors (Netea et al., 2006), yet in this paper, it is suggested that *C. albicans* recognition by epithelial cells has important differences from the recognition by immune cells. Understanding the differential recognition of mucosal colonization versus invasion by the mucosal immune cells remains a challenge to be answered in the future.

Despite these remaining challenges, the study reported by Moyes and colleagues is a major step in understanding the pathways through which the organism has evolved tolerance toward harmless colonizing yeasts while vigorously responding to invasion by *C. albicans* hyphae. This should provide an impulse in the near future to harness this knowledge for both deciphering the remaining questions and using this knowledge for designing novel approaches for the therapy of mucosal *C. albicans* infections.

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REFERENCES

Jawhara, S., Thuru, X., Standaert-Vitse, A., Jouault, T., Mordon, S., Sendid, B., Desreumaux, P., and Poulain, D. (2008). J. Infect. Dis. *197*, 972–980.

Lo, H.-J., Köhler, J.R., DiDomenico, B., Loebenberg, D., Cacciapuoti, A., and Fink, G.R. (1997). Cell *90*, 939–949.

Moyes, D.L., Runglall, M., Murciano, C., Shen, C., Nayar, D., Thavaraj, S., Kohli, A., Islam, A., Mora-Montes, H.M., Challacombe, S.J., and Naglik, J.R. (2010). Cell Host Microbe *8*, this issue, 225–235.

Netea, M.G., Brown, G.D., Kullberg, B.J., and Gow, N.A. (2008). Nat. Rev. Microbiol. *6*, 67–78.

Netea, M.G., Gow, N.A., Munro, C.A., Bates, S., Collins, C., Ferwerda, G., Hobson, R.P., Bertram, G., Hughes, H.B., Jansen, T., et al. (2006). J. Clin. Invest. *116*, 1642–1650.

Perlroth, J., Choi, B., and Spellberg, B. (2007). Med. Mycol. *45*, 321–346.

Romani, L. (2004). Nat. Rev. Immunol. *4*, 1–23.

Standaert-Vitse, A., Sendid, B., Joossens, M., François, N., Vandewalle-El Khoury, P., Branche, J., Van Kruiningen, H., Jouault, T., Rutgeerts, P., Gower-Rousseau, C., et al. (2009). Am. J. Gastroenterol. *104*, 1745–1753.

Weindl, G., Naglik, J.R., Kaesler, S., Biedermann, T., Hube, B., Korting, H.C., and Schaller, M. (2007). J. Clin. Invest. *117*, 3664–3672.